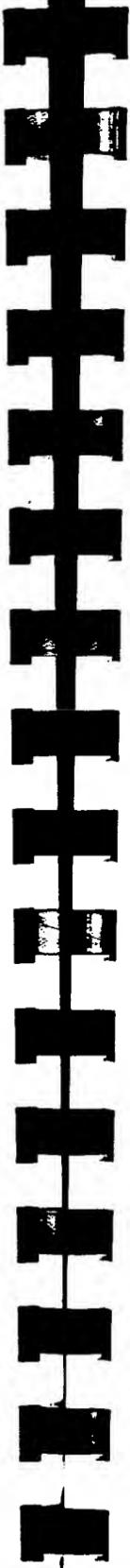


# **EXHIBIT 38**



**Nonisotopic DNA  
Probe Techniques**

# Nonisotopic DNA Probe Techniques

Edited by

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*Front cover photograph:* Color enhanced digitized image of a DNA sequence obtained using the chemiluminescent substrate CSPD to visualize bound alkaline phosphatase conjugate. This illustration was kindly provided by Irena Bronstein and Chris Martin of Tropix, Inc.

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# 8

## Detection of Horseradish Peroxidase by Colorimetry

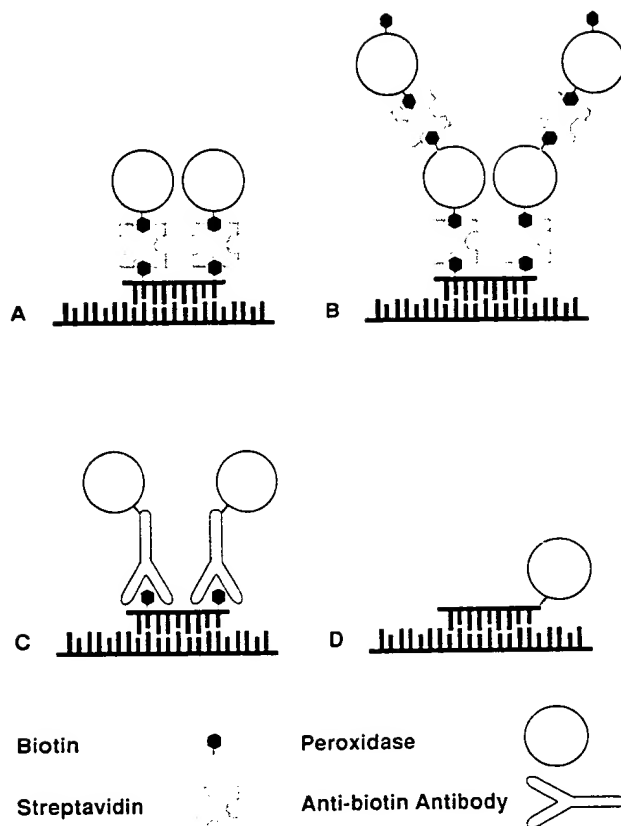
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Department of Investigative Dermatology  
The Rockefeller University  
New York, New York

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### I. INTRODUCTION

#### A. Historical Overview

Horseradish peroxidase (HRP) has been used extensively as a colorimetric marker in biological studies. A hemoprotein with a molecular weight of 40,000, HRP is an ideal detection reagent because of its stability, high turnover rate, and the availability of a wide assortment of colorimetric substrates. Although radioactive techniques are generally more sensitive, the sensitivity of peroxidase detection systems is adequate for many applications, and speed and safety considerations make peroxidase detection



**Fig. 1** Detection of nucleic acid probes with horseradish peroxidase. (A) Hybridization with a biotinylated probe, followed by sequential washes with streptavidin and biotinylated peroxidase. (B) Hybridization with a biotinylated probe, followed by a single wash with a preformed complex of streptavidin and biotinylated peroxidase. (C) Hybridization with a biotinylated probe, followed by detection with anti-biotin antibodies coupled to peroxidase. This technique also applies to detection of probes modified in other ways, such as by sulfonation. (D) Hybridization with a probe that is directly coupled to peroxidase.

been used for a variety of clinical diagnostic tests. Peroxidase detection has also been used for genetic testing applications, such as Y chromosome aneuploidy (Guttenbach and Schmid, 1990).

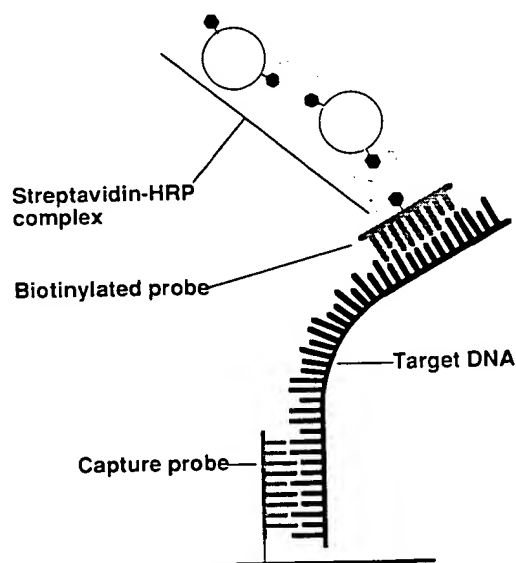
## 2. Sandwich Hybridizations

Sandwich assays are particularly well suited to peroxidase detection. These assays generally include a capture probe that is bound to a fixed matrix, such as nitrocellulose membranes (Dunn and Hassell, 1977; Ranki *et al.*, 1983), microtiter wells (Dahlen *et al.*, 1987; Keller *et al.*, 1989), or

beads (Langdale and Malcolm, 1985; Polsky-Cynkin *et al.*, 1985). The target nucleic acid molecule hybridizes to the capture probe and is thereby bound to the matrix, while a second probe that is either directly or indirectly labeled with peroxidase hybridizes to an adjacent sequence on the target (Fig. 2). These techniques are particularly useful for the detection of polymerase chain reaction (PCR)-amplified nucleic acids, and have been used in assays for human immunodeficiency virus (HIV) (Kemp *et al.*, 1990; Keller *et al.*, 1989),  $\beta$ -thalassemia (Saiki *et al.*, 1988, 1989), and sickle-cell anemia (Saiki *et al.*, 1988) among others. Peroxidase detection is useful for these types of assays because of the high turnover rate of the enzyme, and because of the availability of a number of sensitive substrates for soluble assays.

### 3. Membrane Hybridizations

Peroxidase detection is perhaps less well suited to membrane hybridization techniques. The sensitivity of peroxidase detection does not match that of radioactive systems, although detection of subpicogram bands on Southern hybridizations have been claimed (Sheldon *et al.*, 1986, 1987). The lower stability of peroxidase as compared to alkaline phosphatase



**Fig. 2** Sandwich assays. The target hybridizes to an immobilized probe and is captured to the solid matrix. A biotinylated probe hybridizes to adjacent sequences on the target and is detected by streptavidin-HRP.

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